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STUDIES ON LARVAE OF STRIGEOID TREMATODES FROM THE WOODS HOLE, MASSACHUSETTS REGION[1]

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The superfamily Strigeoidea Railliet, 1919 was erected to contain the trematodes formerly included in the families Holostomidae E. Blanchard, 1847 and Hemistomidae Brandes, 1888. *Holostomum* Nitzsch, 1819 and *Hemistomum* Diesing, 1850 were suppressed as synonyms, respectively, of *Strigea* Abildgaard, 1790 and *Alaria* Schrank, 1788. These worms were known to Goeze and Rudolphi and are common parasites of birds and mammals. Adults live in the intestine of turtles, crocodiles, birds and mammals. The first intermediate hosts are snails and the cercariae are produced in slender sporocysts. The cercariae have forked tails, penetrate their next hosts and occur as metacercariae in snails, leeches, fish, amphibians and rarely in snakes, birds, and mammals. In members of the genera *Strigea* and *Alaria,* an additional stage, the mesocercaria, may be interposed between the cercaria and the metacercaria and these species have a four host life cycle.

Knowledge concerning the life-cycles of these trematodes was retarded for fifty years by the erroneous belief that they differed essentially from other groups. Von Linstow (1877) embryonated eggs of *Holostomum cornucopia* Molin, 1859 [= *Strigea strigis* (Schrank, 1788) Abildgaard, 1790]. He reported that the "embryo" which emerged, transformed, without sporocyst or cercarial generations, into the metacercaria. This organism had been described by Steenstrup (1842) and was designated as Tetracotyle by de Filippi (1854). The metacercariae of the strigeids have characteristic forms which were regarded as genera by the early investigators and designated Tetracotyle, Tylodelphys, Diplostomum, Conocephalus, *et al.* Lenckart (1889) accepted the idea of von Linstow and designated the type of development as "metastatic," *i.e.*, intermediate between the monogenetic and digenetic life cycles of other trematodes. The error was dispelled when Lutz (1921) demonstrated that strigeid metacercariae developed from forked-tailed cercariae. The discovery was quickly confirmed; Ruszkowski (1922), Mathias (1922) and Szidat (1924) showed that other strigeids have furcocercous cercariae. Szidat (1929) described larval development in the strigeids and showed that the "holdfast" or tribocytic organ is a new structure, peculiar to the group.

In a monograph of the Strigeida, Dubois (1938) recognized two superfamilies: Strigeides Dubois, 1936 and Cyathocotylides Dubois, 1936. The first contained three families: Strigeidae Railliet, 1919; Diplostomidae Poirier, 1886, and Proterodiplostomidae Dubois, 1936. The Strigeidae included two subfamilies, Strigeinae Railliet, 1919, parasites of birds, and Duboisiellinae Baer, 1938, with a single genus *Duboisiella* Baer, 1938, parasite of mammals. The Diplostomidae contained two subfamilies: Diplostominae Monticelli, 1888, parasites of birds and Alariinae

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Hall and Wigdor, 1918, parasites of mammals. The family Proterodiplostomidae contained three subfamilies: all parasites of reptiles. The superfamily Cyathocotylides contained two families: Cyathocotylidae Poche, 1925, with parasites of both birds and mammals, and Brauninidae Bosma, 1931, parasites of mammals. Dubois (1968, 1970) published a revised "Synopsis des Strigeidae et des Diplostomidae."

LaRue (1957) reviewed the life-cycles and developmental stages of the digenetic trematodes. Basing his determination primarily on homologies in the formation of the excretory system, he proposed a new system of classification in which a new order, Strigeatoidea, included the strigeids, the schistosomes, clinostomes, azygiids, cyclocoelids, brachylaemids, fellodistomids, bucephalids, and renicolids. In all of these families the cercariae lack stylets, have simple membranous excretory vesicles, forked tails, and primary excretory pores located on the tails. Discovery of the life-cycle of *Renicola thaidus* by Stunkard (1964) removed the family Renicolidae from the Strigeatoidea.

Hoffman (1960) compiled a synopsis of Strigeoidea (Trematoda) of fishes and their life-cycles. He recognized four larval groups: Tetracotyle, Diplostomulum, Neascus, and Prohemistomulum. The Tetracotyles are larvae of species in the family Strigeidae and the adults occur in birds; Diplostomulum and Neascus are metacercariae of members of the Diplostomidae which occur in both birds and mammals, while the Prohemistomula are larvae of cyathocotylids that occur in both birds and mammals. The Tetracotyle have lateral pseudosuckers (cotylae) and the tribocytic organ is bilobed. The diplostomes have small, marginal anterior suckers and the tribocytic organ is circular to oval with a median longitudinal vent. Neascus and Prohemistomulum larvae lack accessory suckers. Tetracotyles produce cysts of parasitic origin but the larvae of *Apatemon burti* in leeches do not encyst for five or six weeks and are not infective until encysted (Stunkard, Willey and Rabinowitz, 1941). In the diplostomes the cercariae do not have cystogenous glands and there are no cysts of parasitic origin.

Strigeid trematodes are common parasites of shore birds and Linton (1928) described three species from gulls taken at Woods Hole. One, described as *Proalaria indistincta* (Guberlet) was based on three specimens, two from *Larus argentatus* and one from *Larus atricilla*. A single, damaged specimen from *L. argentatus* was described as *Alaria* species. Three specimens from *L. argentatus*, one from *L. atricilla*, and three from *L. delawarensis* were described as *Strigea bursigerum* (Brandes) Lühe. The first species was identified by Dubois (1970) as *Diplostomum spathaceum indistinctum* (Guberlet, 1923) Hughes, 1929 and the second as *Diplostomum gavium* (Guberlet, 1922) Hughes, 1929. The third species was assigned by Dubois (1968) to *Cardiocephalus mediconiger* Dubois and Vigueras, 1949 [= *Cardiocephaloides mediconiger* (Dubois and Vigueras, 1949) Dubois, 1970].

Although adult specimens of strigeid trematodes are common in shore birds, the larval stages are virtually unknown in marine snails. Cable (1956) described two cyathocotylid species: *Cercaria caribbea L.* and *Cercaria caribbea LI.* from species of *Cerithium* in Puerto Rico. Hutton and Sogandares-Bernal (1959, 1960) described cyathocotylid larvae from *Cerithium muscarum* which encysted in the muscles of mullets, *Mugil* spp., and became sexually mature in the intestine of

the brown pelican, *Pelicanus occidentalis,* the black crowned night heron, *Nycticorax nycticorax,* and the opossum, *Didelphis virginianus.* The adults were identified as *Mesotephanus appendiculatoides* (Price, 1934) Lutz, 1935. A field caught gull, *L. delawarensis,* and a raccoon, *Procyon lotor,* yielded specimens of *M. appendiculatoides* when fed infected mullet flesh. Another life-cycle was reported by Martin (1961) who found sporocysts and cercariae in *Cerithidea californica,* encysted metacercariae in the muscles of *Fundulus parvipinnis* and *Gillichthys mirabilis,* and adults were obtained experimentally in chicks. The worms were identified as *Mesostephanus appendiculatus* (Ciurea, 1916) Lutz, 1935. The species was described originally from the intestine of dogs and cats that had been fed metacercariae encysted in the muscles of various fishes, *Tinca tinca, Aspius aspius, Blicca bjorkna,* and *Carassius carassius,* taken from the Danube River in Roumania. Dubois (1953, page 108) stated that the natural hosts are pelicans and "*Mesostephanus appendiculatus* Ciur. et *M. longisaccus* Chdl. sont des parasites secondaries ou erratiques du chien." Leonov (1958) reported *M. appendiculatus* from *L. argentatus* in Russia and Yamaguti (1971) from *L. delawarensis* in the United States.

With the exceptions of the cyathocotylids mentioned, the only other strigeid larva reported from a marine snail is *Cercaria nassa* Martin, 1945, from *Ilyanassa obsoleta* (syn. *Nassa obsoleta*) taken in the Woods Hole region. The incidence of infection was very low, averaging 0.1 per cent. The cercariae developed in long slender sporocysts, and both sporocysts and cercariae were figured. In addition to the morphology of the cercariae, Martin (1945) reported on their behavior and swimming activity. The cercariae are not responsive to light, are uniformly distributed in the water, and swim with the tail in advance. They manifest alternate periods of activity and rest. At rest the cercaria is suspended in the water, tail uppermost with the furcae spread at an angle of approximately 90 degrees. It slowly sinks until it suddenly darts upward in a spiral course, caused by a sculling movement of the tail, which ends with the body in a horizontal position. As the larva sinks, the body turns downward and after it reaches a vertical position the next upward dash is started. Martin (1945) measured the length of the swimming and inactive periods; the average of 40 observations gave the following data: resting, 7.87 seconds, swimming, 2.08 seconds. As the cercariae grow older the swimming period becomes shorter and the resting period is lengthened. Consequently, the cercariae tend to lie deeper in the water. Martin recognized the cercaria as a strigeid species and compared it with *Cercaria flexicorpa* Collins, 1935. Attempts were made to determine the second intermediate host and common fishes were exposed to the cercariae. The larvae attached by their anterior organs to *Fundulus* spp. and *Paralichthys dentatus* and immediately shed their tails. They remained attached to *Fundulus* for several hours but seemed unable to penetrate and eventually dropped off. They penetrated the thin web of the fin of *P. dentatus* but disintegrated after a few hours.

Hunter and Vernberg (1960) at the Duke University marine laboratory, Beaufort, North Carolina, reported the finding of strigeids in laboratory reared birds, *Rhynchops nigra, Sterna hirundo,* and *Sterna albifrons,* that had been fed small unidentified fishes. Dissection disclosed large numbers of metacercariae, some encysted, in the ventricles and frequently in the eyes of *Menidia menidia* and

Mugil cephalus. Feeding experiments conducted in 1956 yielded worms of different ages and degrees of development when young *M. cephalus* were fed to *R. nigra*. Feeding experiments were continued in 1959. Metacercariae from the brain of *M. menidia* were fed to two *R. nigra* and one *S. hirundo*. The results were negative and the authors concluded that the metacercariae were not infective before encystment. Whole brains were fed to four, one-day old, chicks. Three received 20 and the other 30 brains. Five worms were recovered from one chick after 72 hours and eleven worms from another after seven days. The other two chicks were negative on examination. Worms were submitted to Dr. Dubois and identified as *Cardiocephalus medioconiger* [= *Cardiocephaloides medioconiger* (Dubois and Vigueras, 1949) Dubois, 1970]. Dubois (1970, page 722) reported, "*Cardiocephalus* Szidat, 1928 tombe comme homonyme de *Cardiocephalus* Broili, 1904 (Amphibia: Lepospondyli: Microsauria), type *sternbergi (Permien*, Texas)."

The authors noted that *Cercaria nassa* occurs frequently in *Nassarius obsoleta* in the Beaufort, North Carolina area. They recalled that Martin (1945) had recognized *C. nassa* as a strigeid but had failed to obtain infection in species of *Fundulus* and *Paralichthys dentatus*. They confirmed the results with *Fundulus* spp. However, they reported penetration into small *M. cephalus* and recovery of larvae at intervals of ten hours and 18 days, although the locations were not given. Attempts at penetration of the general body surface of fishes were unsuccessful, but penetration through the roof of the mouth was observed and entrance through the gills was suspected. Encysted metacercariae were found only in the brain, but there was no assurance that the worms resulted from experimental exposure. There is no evidence that *C. nassa* is the larval stage of *C. medioconiger* and such uncritical speculation may lead to unfortunate errors like the one made by Hunter and Vernberg (1953) where the cercaria of *Zoogonus lasius* (Leidy, 1891) was identified as the larval stage of *Gynaecotyla adunca* (Linton, 1905).

An observation by Abbott (1968) has impelled renewed study of *C. nassa*. He reported metacercariae of an unidentified trematode on the brain of *Fundulus heteroclitus*, representing 100% infection of a sample of fishes obtained from the Chesapeake Biological Laboratory, Solomons, Maryland. Although the author was concerned with the function of the pineal gland of the fish, the description and figures of the parasite identify it as a strigeid. Since *C. nassa* is the only described strigeid cercaria from marine snails on the Atlantic coast and is sympatric with *F. heteroclitus*, the possibility appeared that it might be a stage in the life cycle of the metacercariae in the brain of that species. This possibility has been under investigation during the past three summers and a preliminary report was presented at the annual meeting of the American Society of Parasitologists in August, 1971, at Los Angeles, California.

Materials and Methods

During the past three years over 10,000 specimens of *I. obsoleta* have been collected and examined for infection with *C. nassa*. They came from various locations on the seashore near Woods Hole, Massachusetts and from beaches on adjacent Martha's Vineyard, Elizabethan and Weepecket Islands. For ease in computation, they were isolated ten in a bowl and the water was changed daily,

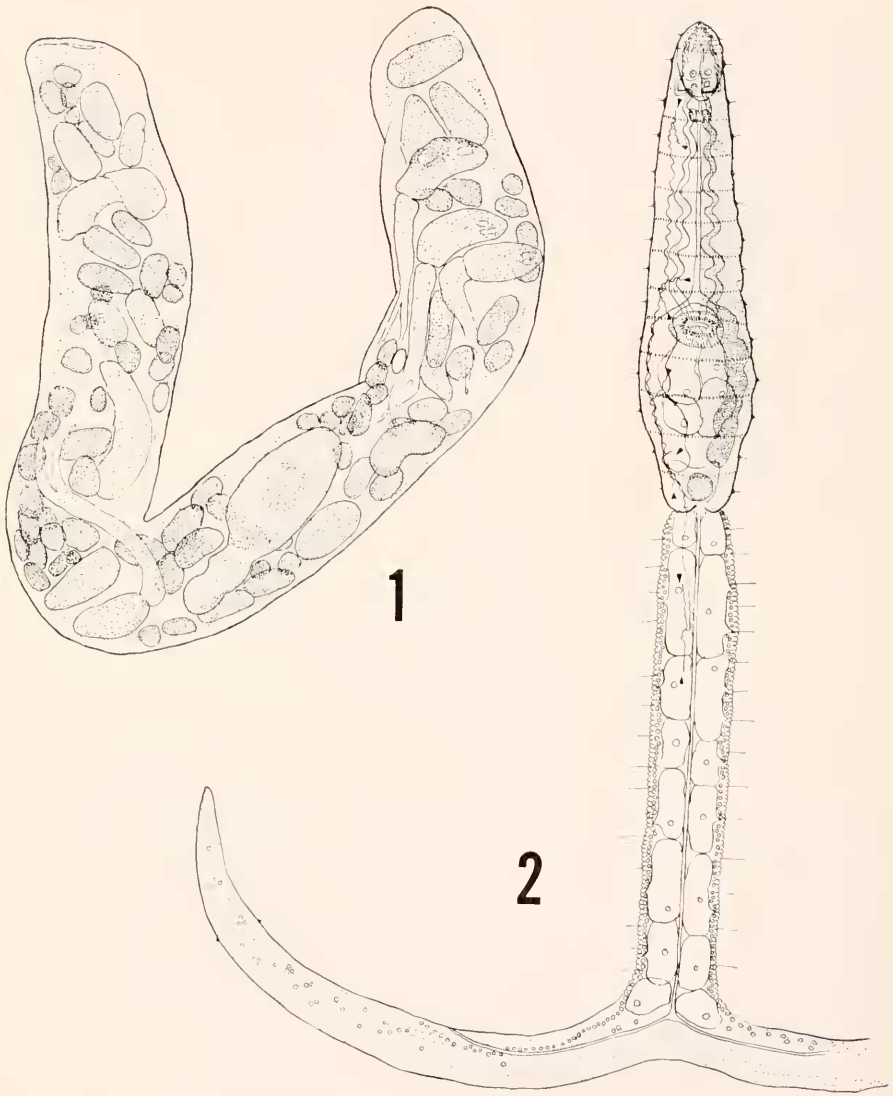

FIGURE 1. Sporocyst of *C. nassa;* specimen 2.20 mm long, 0.20 mm wide. For clarity, less than one-half of the germ balls and cercariae are represented.

FIGURE 2. *Cercaria nassa;* a composite drawing made from pencil sketches of living specimens and study of fixed and stained ones.

sometimes in the morning and sometimes in the late afternoon to check the time of cercarial emergence. When the snails were transferred to a clean bowl, the water from which they were taken was examined for cercariae. Other species, *e.g.,* cercariae of *Zoogonus lasius, Himasthla quissetensis, Lepocreadium setiferoides, Stephanostomum tenue* and *Stephanostomum dentatum* were common while those of *Gynaecotyla nassicola* and *Microbilharzia variglandis* were rare.

After one week to ten days, if no *Cercaria nassa* appeared, sample snails were crushed for examination and others were discarded. As a rule, when snails are brought into the laboratory, with a somewhat higher temperature, they shed readily and they will continue to shed if well fed, but starved snails soon fail to liberate cercariae although the infection persists and shedding can sometimes be induced by feeding the snails. It is clear the reproduction of the parasite is inhibited when the host is unnourished. Shedding of *C. nassa* is unpredictable; some days scores of cercariae emerged followed by several days with few or no cercariae and then a renewal of shedding. The larvae emerged during both day and night but most were shed in the morning hours. Whether or not cercariae are infective immediately after release or only after a period of acclimatization, and for how long a time, are unknown. The cercariae lose vitality after 24 hours and may no longer be infective. When fishes are left in bowls with shedding snails, the number of cercariae shed is unknown, but larvae of different ages and different degrees of infectivity are present.

During the summer of 1969, 2,740 specimens of *I. obsoleta* were isolated and no infections with *C. nassa* were found. In 1970, 3,980 snails were isolated and a collection of 630 snails, taken July 8, in Squiteague Bay, near North Falmouth, yielded three infections, the only ones discovered during the summer. In 1971, 3,590 snails were isolated with negative results, but a single infected specimen was provided by Dr. Paul Krupa who found it in a collection made on Penzance in Great Harbor, Woods Hole. In the past three years, only four of more than 10,000 snails shed *C. nassa*. The incidence of infection in the Woods Hole region is less than that found by Martin some thirty years ago. The snails are as numerous, so the final hosts must be more rare.

On July 17, 1969, Dr. Langley Wood brought about 100 specimens of *Fundulus heteroclitus* taken near the Chesapeake Biological Laboratory, Solomons, Maryland and one week later sent 200 specimens of *Nassarius vibex* from the same area. The fishes were dissected but no strigeid metacercariae were found. Dr. Wood reported that *I. obsoleta* is rare in Chesapeake Bay and is replaced there by *N. vibex*. One of the *N. vibex* shed *C. nassa*. The snail continued to shed cercariae during the summer, sometimes at intervals of several days and was crushed on September 15th. There had been no cercariae for almost three weeks, but the haemocoele was filled with active sporocysts of all sizes. On July 21, 1970, Dr. Victor Sprague sent 200 specimens of *N. vibex* from the Chesapeake Laboratory, but there were no infections with *C. nassa*.

Strigeid trematodes are predominantly parasites of birds and it seemed likely that the adult stage of *C. nassa* would be found in birds that feed on *Fundulus* spp. Accordingly, when an infection by *C. nassa* was found in 1970, Dr. Norman Sinclair and Mr. Peter Oldham collected two nestling snowy egrets, *Egretta thula*, and one black-crowned night heron, *Nycticorax nycticorax*, from the rookery on Martha's Vineyard Island and three cormorants, *Phalacrocorax auritus*, and three gulls, *Larus argentatus*, from the rookery on the Weepeckets. Repeated fecal examinations showed no previous infection by trematodes and the birds were maintained on commercial food to assure against accidental infection. One cormorant and one gull were lost, but the other birds survived and were autopsied in in September.

Experiments and Results

In 1969, when the specimen of *N. vibex* was found to be liberating *C. nassa*, experiments were begun to determine whether or not the metacercariae would attack and develop in *Fundulus* spp. Fishes, provided by the Supply Department of the Marine Biological Laboratory, were placed in bowls with swimming cercariae and although penetration was not observed, the presence of discarded tails on the bottom of the bowls indicated that the bodies of the cercariae had entered the tissues of the fish. Dissection of fishes that had been exposed yielded metacercariae on the surface of the brain. The larger worms were active, not encysted, and fell off the brain-surface when the cranial cavity was opened under sea water. They were not found in the gills, eyes, or optic nerves. The smallest specimen was found on August 5, in a fish that had been exposed on August 1. This specimen was lost. Two other fishes, exposed on August 1, were dissected on August 16. Four metacercariae were recovered; a fixed and mounted specimen (Fig. 3) measures 0.325 mm long and 0.24 mm wide. A fish dissected on September 8, yielded a metacercaria that measured 0.44 by 0.315 mm. Two fishes dissected on September 9 yielded three metacercariae; the largest fixed and mounted, (Fig. 6) measured 0.70 by 0.63 mm. It was very active, with the edges of the body extending and retracting constantly. In it the reticular reserve excretory system was filled with concretions. The purpose of the study was to obtain adult specimens rather than to trace development in the fish, so few fishes were dissected. It was hoped to feed the metacercariae to baby chicks at the end of the summer, but baby chicks are not available in September in the Woods Hole area. Eighteen fishes that had been exposed were decapitated, the brains were removed and fed to a pigeon on September 12. The bird was autopsied on September 18, but the results were negative.

When snails infected with *C. nassa* were found on July 8, 1970, experimental infection of *Fundulus* spp. was renewed and efforts were made to complete the life-cycle by the use of fish-eating birds. The rookeries on Martha's Vineyard and the Weepeckets provided nestlings as noted earlier.

On July 12, two fishes were placed in a bowl with hundreds of cercariae that had been liberated by the three snails. One of the fishes died the next day and the other on the following day. Whether or not these deaths were the result of the massive exposure is uncertain, but large numbers of cercariae must have penetrated the tissues of the fishes because there were hundreds of detached and discarded tails on the bottom of the bowl. In later exposures, the fishes were not subjected to heavy infection; rather, repeated exposures were made with fewer cercariae. After repeated exposures, the fishes were maintained in larger aquaria. For exposure, usually two fishes were placed in a bowl with the cercariae that had emerged during the previous 24 hours, some 20 to 50 larvae. As fishes that had been exposed died during the summer, they were dissected to follow the development of the metacercariae. Exposures were terminated about the middle of August and thereafter the cercariae were studied for morphological details. On August 25, one snail was crushed to obtain sporocysts and developing cercariae. The digestive gland was heavily infected with active sporocysts although for the past two weeks the number of cercariae liberated had been small. A second infected

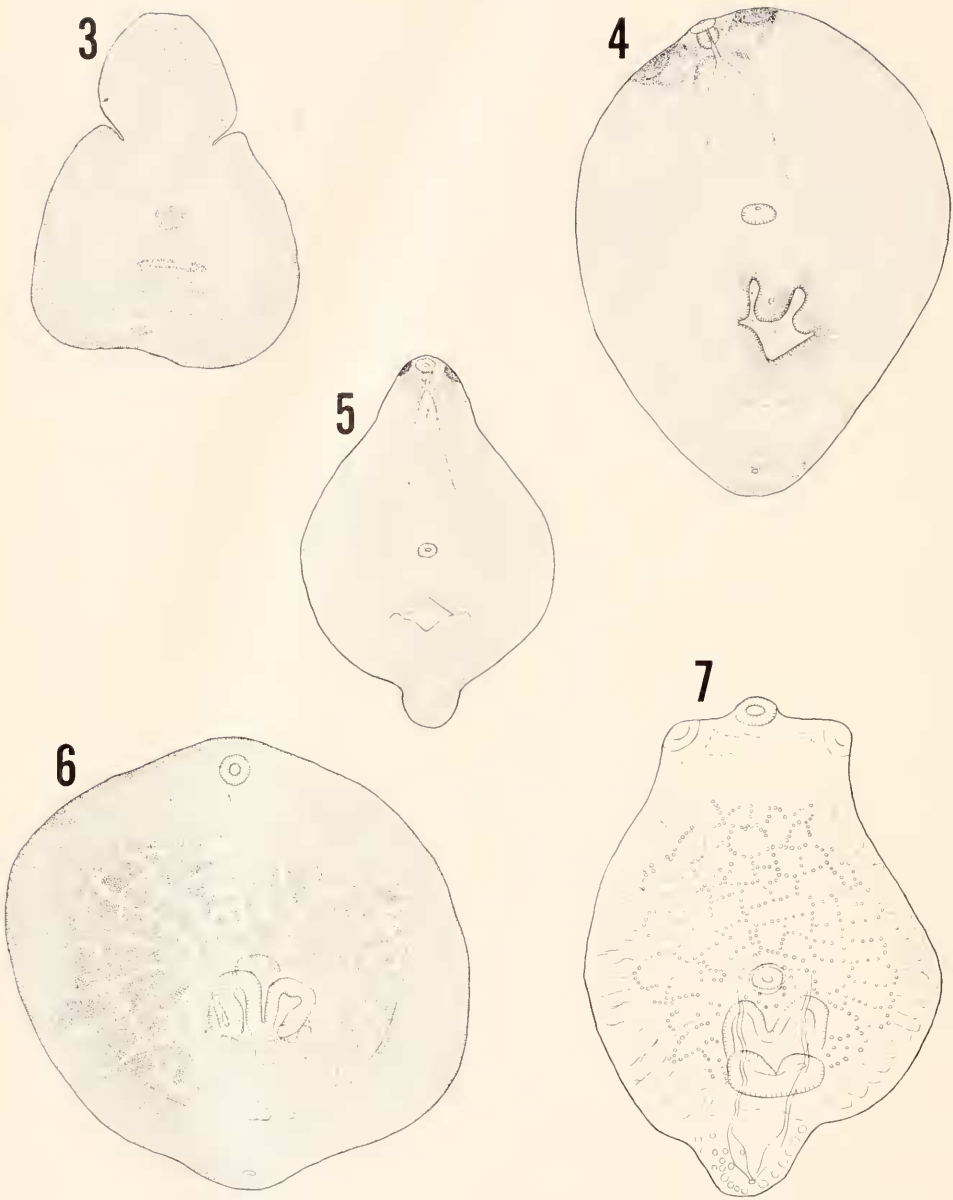

FIGURE 3. Metacercaria from brain of *F. heteroclitus;* taken 11 August, 1969. Fixed and stained, it is 0.325 mm long, 0.24 mm wide and shows cellular aggregates of metamorphosis.

FIGURE 4. Metacercaria taken 15 September, 1970; fixed and stained, is 1.25 mm long, shows outlines of future organs, bilobed tribocytic organ, and beginning of hindbody.

FIGURE 5. Metacercaria taken 15 September, 1970; fixed and stained, is 1.30 mm long with distinct hindbody.

snail was crushed on August 28 and the third on September 3. Both harbored heavy infections.

On September 13, six fishes, exposed repeatedly since mid-July, were fed to each of the experimental birds. On September 15, a lone remaining fish was dissected and seven metacercariae (Figs. 4, 5) were recovered from the surface of the brain. None of the metacercariae was encysted. The two white egrets and the black-crowned night heron were autopsied on September 19, the two cormorants on September 20, and the two gulls on September 21. No trematodes were found in any of the birds. The larvae taken from the fish on September 15 were active, but worms at that stage of development may not be sufficiently mature to survive in the digestive tracts of piscivorous birds.

The single infected snail found by Dr. Paul Krupa on July 21, 1971 liberated cercariae during the summer. Specimens of *Fundulus heteroclitus* were placed in bowls with swimming cercariae as in previous seasons and 28 fishes were exposed. One fish was dissected on August 18, and a single unencysted strigeid metacercaria was found on the brain. It was hoped to feed the metacercariae to day-old chicks and ducks at the end of the summer, but again baby birds were not available. Accordingly, the fishes were held in aquaria of the U.S. Bureau of Fisheries through the courtesy of Mr. Charles Wheeler and in the Marine Biological Laboratory through the courtesy of Mr. John Valois. Six fishes survived in the M.B.L. Day-old chicks were provided by the M.B.L. on June 29, 1972. The fishes were dissected on June 29 and June 30. One fish had two encysted larvae on the brain and another had a single encysted metacercaria between the brain and the cranial wall. The other fishes were negative. The cysts were oval with firm, rather thick walls. The fishes had been isolated over the winter with no opportunity for reinfection, so the parasites were unquestionably carried over from the previous summer. The finding of encysted larvae shows that the tetracotyles ultimately encyst. The encysted larvae were fed to a chick but no worms were found when the bird was autopsied six days later. The failure to obtain infection in the chick is not surprising since only occasionally, or rarely do strigeids persist in chicks.

Experiments conducted over a period of three years have shown that *C. nassa* is not the larval stage of the metacercariae on the brain of *Fundulus* spp., but have not disclosed the final host and adult stage of those metacercariae.

Observations

The metacercariae from the brain of *Fundulus* spp. are obviously members of a single species. They grow and develop on the surface of the brain but encysted specimens have been observed only after a long interval. The smallest were flattened, circular to oval, and about 0.2 mm in diameter. They were immobile, unattached, and floated free when the meniges were removed by dissections in sea water. In these specimens, the cercarial structures were completely obliterated and the larvae consisted of closely packed nuclei, with little or no cytoplasm and no

Figure 6. Metacercaria taken 9 September 1969; fixed and stained, is 0.70 by 0.63 mm. It was very active when alive; the figure shows the suckers, developing pseudosuckers, bilobed tribocytic organ, excretory pore and outline of the excretory tubules.

Figure 7. Drawing made from pencil sketches of living metacercaria to show the concretions in the excretory tubules and the spaces of the reserve bladder.

distinct cell boundaries. The first recognizable features of development were aggregations of nuclei at the locations of the future suckers, the tribocytic organ, and the reproductive organs. The development of the metacercariae involves a complete metamorphosis, comparable to that described by Szidat (1929) in the life-cycle of *Cotylurus cornutus* (Rudolphi, 1808), (syn. *Tetracotyle typica* Diesing, 1858). He compared the metamorphosis of strigeid trematodes with that of certain insects, "Entwicklung mit Umwaldung" and stated p. 668, "Es ist dies Verhalten der Larven der klare Ausdruck einer Metamorphose, und zwar, nach den bestehenden Begriffen, einer holometabolen Metamorphose der Cercarien, wie wir sie bisher bei Trematoden nicht gekannt haben." In a specimen taken August 16, 1969, stained and mounted, (Fig. 3), the oral sucker, acetabulum and reproductive complex are represented by cellular aggregates and the tribocytic organ is forecast by a shallow transverse depression. A more developed specimen taken September 9, 1969, somewhat flattened (Fig. 6) shows the suckers, the cellular condensations that form the lateral pseudosuckers, the bilobed tribocytic organ and the outline of the network formed by the excretory system. Fixed, stained and mounted it is 0.70 mm long and 0.63 mm wide. Figures 4 and 5 were made from two of seven larvae taken September 15, 1970; they measure 1.25 and 1.30 mm respectively in length, and show the dorso-posterior protrusion that becomes the hindbody of the adult. Figure 7 is from pencil sketches of a larva to show the reticular pattern of the excretory system as outlined by rows of concretions and by the spaces of the reserve system at the periphery of the body.

The description of *Cercaria nassa* by Martin (1945) is brief and not entirely correct. He described six penetration glands, but there are only two pairs. A more complete account is presented.

The haemal sinuses of the digestive gland of an infected snail may be filled by hundreds of tangled sporocysts. The snails harbored natural infections and accordingly, only daughter sporocysts were present. They vary in size from small, cylindrical to fusiform specimens, 0.25 mm in length and 0.06 mm in width with a few small germ-balls to large gravid individuals, 3 mm in length and 0.10 to 0.20 mm in width, with hundreds of germ-balls and developing cercariae, (Fig. 1). The increase in length is relatively greater than the increase in width. In general, the length is 10 to 15 times the width of a sporocyst. The ends may be conical, extended and tapering, blunt and flattened, or slightly concave. The ends, especially the anterior end, are more muscular than the rest of the body and have a concentration of nuclei in their walls. Often one or both ends may be rounded and knob-like, separated from the remainder of the body by a constricted, neck-like region. The body wall contains circular, longitudinal and diagonal muscle fibers and young sporocysts are very active. They may proceed with either end in advance. They may be nematoform, or contractions of circular muscles may produce constrictions with accompanying protuberances and bizarre shapes. With increase in size and number of progeny, mobility is reduced and the larger sporocysts are relatively inert. The body wall is covered by a very thin, transparent membrane which is often raised in fixed and stained specimens.

The germ balls are almost spherical until they attain a diameter of 0.03 to 0.04 mm when they become oval and continue to increase in the long axis. At a length of approximately 0.05 mm, the constriction that denotes the tail appears, and as

length increases the bifid character of the tail becomes more pronounced. Further development leads to the fully formed cercariae which leave the sporocyst through a birth pore, situated near the anterior end. The pore is not recognizable in fixed and stained specimens. The increase in number of sporocysts, and the presence of large numbers of small individuals in snails that have been isolated for several months and have continued to liberate thousands of cercariae, strongly suggests more than one generation of sporocysts. The recognition of a daughter sporocyst within a mother sporocyst is impossible, since there are no distinguishing features to discriminate between a daughter sporocyst and a cercaria at that stage of development.

The cercaria

In living specimens under slight coverglass pressure the body measures 0.06 to 0.26 mm in length and 0.03 to 0.05 mm in width. The tail-stem is about as long and as wide as the body; the furci somewhat shorter but very extensile. Each region is capable of independent extension and contraction. The acetabulum, situated in the posterior half of the body, is about 0.02 mm in diameter; the "anterior organ" is oval, 0.033 to 0.040 mm in length and 0.018 to 0.022 mm in width; the pharynx measures 0.008 to 0.009 mm in diameter and is separated from the anterior organ by about the same length. The body and tail bear papillae, each surmounted by a single seta. On the body they alternate with the rows of cuticular spines; on the tail-stem and furci the number and position apparently are not constant. In specimens killed in hot water, fixed in Duboscq-Brasil solution, stained and mounted (Fig. 2), average measurements are body-length, 0.165 mm; width, 0.044 mm, greatest in the posterior half of the body. The tail-stem has almost parallel sides, averages 0.18 mm long and 0.036 mm wide; the furci 0.135 mm long. The surface of the body but not of the tail-stem, bears cuticular spines, largely disposed in annular rows, eight in the preacetabular and four in the postacetabular portion of the body. Contractions of circular muscles at the level of the cuticular spines may give the body a crenate appearance. There are small spines along the edges of the furci. There are two rows of alternating spines around the opening of the acetabulum.

The structure called the "oral sucker" or "anterior organ" is a conspicuous feature of strigeid and schistosome cercariae. Hoffman and Hundley (1957) referred to it as the "penetration organ." It is not an oral sucker although after metamorphosis, its remains are reorganized to form the definitive oral sucker of the adult. The term "anterior organ" is a meaningless and unsuitable designation for this organ, which by its structure and function is adapted to penetrate the tissues of the next host and transmit the secretions of the penetration glands into the resulting wound. For it I propose the name "penetrantorium." The name "perforatorium" would be equally appropriate but since the term has been applied to the acrosome of the spermatozoan, its use would not be approved by semantic purists like the late Dr. Libbie Hyman who maintained that only homologous structures should bear the same appellation. An example of incongruity is the term, acetabulum, which denotes very different structures in digenetic trematodes and vertebrates.

In *C. nassa* the penetrantorium is oval, protrusible and retractile, working with a piston-like movement. It is retracted by fibers that originate more posteriorly from the body-wall and are inserted on the posterolateral aspects of the organ, and on retraction the anterior tip of the body becomes cupuliform. The penetrantorium is driven forward explosively by rapid, successive contractions of circular muscles from the acetabulum to the anterior end of the body. It contains four large secretory cells, two on each side, one dorsal the other ventral. The nuclei, 0.004 to 0.005 mm in diameter, are located in the posterior portions of the cells, the nuclei of the ventral cells posterior to those of the dorsal cells. The function of these cells is obscure; the secretion may contain enzymes of use in penetration, or it may serve to maintain rigidity of the organ and make the forward thrust more effective. When protruded, the anterior tip bears a battery of forward directed spines, typically arranged in five alternating rows, with each spine below and between the two above it. The arrangement varies with muscular contractions of the tip; the most common one presents a dorsal row with 2 spines, the next 3 spines, the next 4 spines, the next 5 spines, and the lowest row above the mouth has 4 spines. In other specimens the number may vary to a condition where the rows have 4, 5, 6, 5, and 4 spines in successive rows. Surrounding the anterior terminal spines, there is a small glabrous circular area and the body wall is then encircled by a band of closely set, alternating spines, typically arranged in eight rows. There is a zone of scattered cuticular spines between this zone and the previously described first annular ring of cuticular spines. The prepharygeal portion of the digestive tract passes forward in the center of the penetrantorium and the ducts of the penetration glands enter on the posterolateral borders, two on each side, extend through the organ, and open on the anterior face, on either side of the mouth.

There are four penetration glands, situated in the intercecal area between the acetabulum and the excretory bladder. Their ducts, two on each side, pass anteriad along the dorsolateral faces of the acetabulum, dorsal to the anterior ends of the digestive ceca and enter the posterolateral faces of the penetrantorium. In their initial portion, they contain particulate matter but in the terminal portion the contents are fluid and stain with vital dyes. If the penetrantorium and anterior end of the body are retracted, the ducts frequently buckle and become dilated before their entrance into the penetrantorium.

The digestive system is well developed. The mouth is situated on the anterior aspect of the penetrantorium and the canal passes through this structure to emerge as a prepharygeal section, about as long as the pharynx before opening into that organ. The esophagus is long, bifurcating anterior to the acetabulum. There are short lateral extensions, lined with cuticula, which open into the digestive ceca. The ceca are lined with a layer of epithelial cells and terminate at the level of the excretory vesicle.

The flame-cell formula of the excretory system is $2[(2 + 2) + (2 + 2) + (2)]$. There is no transverse commissure between the collecting tubules of the two sides. The lateral collecting tubules contain long cilia; they fuse to form the excretory vesicle, a trefoil shaped sac with a larger, median posterior lobe. They separate in the base of the tail and surround the "island of Cort" before they unite again to form the central canal in the tail. At the distal end of the tail-stem, they separate again and enter the furci, opening on the anterior faces about one-fourth of the

distance to the furcal tips. In the body the most anterior flame-cell is ventral in position, the next one is dorsal and they alternate posteriorly. Of the flame cells in the tail, the anterior pair is dorsal and lateral, the posterior pair is ventral and median.

The wall of the tail consists of external circular, median longitudinal and inner diagonal fibers and the core is filled with the "caudal bodies" or "glycogen cells," usually arranged in eight pairs, which are attached to the central excretory canal. These cells become filled as the cercaria matures in the haemal sinuses of the snail; they are depleted and shrink as the cercaria swims by lashing of the tail. According to Ginetsinskaya and Dobrovol'skii (1962; English translation 1968, page 7), "The number and form of the caudal bodies vary in cercariae of the same species and depend upon age. As the glycogen in them is expended, the large caudal bodies take on an irregular, stellate form and then become inconspicuous."

The future gonads and ducts are represented by a cluster of deeply staining cells situated at the level of the excretory bladder.

Discussion

Cercaria nassa closely resembles *Cercaria scudderi* described by Olivier (1941) from the pulmonate snail, *Lymnaea palustris elodes* Say, taken in Cheboygan County, Michigan. The two are almost identical in size, have the same number and distribution of penetration glands and flame-cells, but differ in details of spination. Dubois (1966) predicated that *C. scudderi* is identical with the larva of a species described by Hoffman and Hundley (1957) as *Diplostomum baeri eucaliae*. These authors found metacercariae in the brain of the stickleback, *Eucalia inconstans*, taken from a stream at Grand Forks, North Dakota. Fed to chicks, they obtained adults in four to six days. Eggs were embryonated, miracidia emerged and penetrated laboratory-reared snails, identified as *Stagnicola palustris elodes*, and produced sporocysts and cercariae. Infection of sticklebacks completed the life-cycle. The adults were identified as *Diplostomum baeri* Dubois, 1937, but were designated as a new subspecies, *D. baeri eucaliae*. Dubois (1966) recognized it as a distinct species, *Diplostomum scudderi* (Olivier, 1941). The general morphology of *C. nassa*, and especially the absence of commissures in the excretory system, identify it as a species of *Diplostomum* and it is designated as *Diplostomum nassa* (Martin, 1945). Larvae of the genus *Diplostomum* were included in a larval group, Diplostomulum, by Brandes (1892). These larvae occur in the musculature, eyes, brain, and spinal cord of fishes and tadpoles of frogs and toads. Indeed, the genus *Diplostomum* was erected by von Nordmann (1832) to contain *D. volvens*, a metacercaria from the eyes of freshwater fishes that proved to be the larval stage of *Diplostomum spathaceum* (Rudolphi, 1819). Since diplostome metacercariae occur frequently in the brain and eyes of fishes, a possible connection between *C. nassa* and the metacercariae on the brain of *Fundulus* spp. was a logical presumption. Experiments, however, have dispelled the idea.

Study of metacercariae from the brain of *Fundulus* spp. shows that the tribocytic organ is bilobed, and that the openings of the pseudo-suckers (cotylae) are directed medially, not anterior and marginal as in the diplostomes. The development of these larvae establishes their identity as tetracotyles, the characteristic larvae of the Strigeidae. Tetracotyles of species in the genera *Apatemon* and

Cotylurus encyst in mollusks and leeches with adults in aquatic birds. Tetracotyles of these and other species also encyst in vertebrates. They occur in the body cavities, peritoneum, muscles, liver and eyes, but so far have not been reported from the brain.

The larval groups appear to be characteristic of families but specificity of hosts is not closely restricted and mature worms ordinarily do not persist in their host for a long time. Considering this subject, Baer and Joyeux (1961) reported, page 639 "On observe, chez les Strigeida, une corrélation très étroite entre le biotope de l'hôte définitif et celui du deuxième hôte intermédiare, qui assure un degré élevé de spécificité écologique. Cette constation est d'autant plus frappante qu'il est possible d'infester expérimentalement des hôtes très différents en leur faisant ingérer des métacercaires. Par example, des Chiens et des Chats peuvent être infestés par des Trématodes vivant normalement chez des Oiseaux rapaces et des Pigeons, Caille, Poulet, par des Vers d'Oiseaux aquatiques."

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Summary

Metacercarial stages of a strigeid trematode were reported from the brain and eyes of *Menidia menidia* and *Mugil cephalus* at Beaufort, North Carolina by Hunter and Vernberg (1960) and from the brain of *Fundulus heteroclitus* taken in Chesapeake Bay by Abbott (1968). Strigeid metacercariae occur also on the brain of species of *Fundulus* in the Woods Hole, Massachusetts region. The only strigeid cercaria described from the mid-Atlantic coast is *Cercaria nassa* Martin, 1945. Attempts to infect *Fundulus* spp. with *C. nassa* were futile and attempts to infect avian species, egrets, herons, cormorants, and gulls with metacercariae from *Fundulus heteroclitus* gave only negative results. The cercaria and metacercaria are described. *Cercaria nassa* belongs to the larval group, Diplostomulum, and is named *Diplostomum nassa* (Martin, 1945), family Diplostomidae. The metacercaria from the brain of *F. heteroclitus* belongs to the larval group, Tetracotyle, family Strigeidae. Accordingly, the two larvae are members of entirely different groups.

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